



## Short communication

Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in feral cats (*Felis silvestris catus*) in Majorca, Balearic Islands, SpainJavier Millán<sup>a</sup>, Oscar Cabezón<sup>b</sup>, Marcela Pabón<sup>c</sup>, J.P. Dubey<sup>d,\*</sup>, Sonia Almería<sup>c,e</sup><sup>a</sup> Sanitat i Control de Fauna (Wildlife Health and Control), Conselleria de Medi Ambient, Govern de les Illes Balears – Fundació Natura Parc, 07142 Santa Eugènia, Balearic Islands, Spain<sup>b</sup> Servei d'Ecopatologia de Fauna Salvatge (SEFaS), Departament de Medicina i Cirurgia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain<sup>c</sup> Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain<sup>d</sup> Animal Parasitic Diseases Laboratory, Animal and Natural Resources Institute, Agricultural Research Service, United States Department of Agricultural, Beltsville, MD 20705, USA<sup>e</sup> Centre de Recerca en Sanitat Animal (CRESA), UAB-IRTA, Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

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## ABSTRACT

Felids are important in the epidemiology of *Toxoplasma gondii* infection because they are the only hosts that can excrete environmentally resistant oocysts. Antibodies to *T. gondii* and *Neospora caninum* were determined in serum samples from 59 feral cats (*Felis silvestris catus*) captured in baited traps during authorized predator control campaigns in Majorca, Balearic Islands, Spain. Antibodies to *T. gondii* assayed by the modified agglutination test (MAT) were found in 50 (84.7%) of 59 cats with MAT titers of 1:25 in one cat, 1:200 in four cats, 1:500 in seven cats, 1:1000 in nine cats, and  $\geq 1:2000$  in 29 cats. Seroprevalence (MAT, 1:25 or more) was significantly higher in adults (94.6%) than in juveniles (<6 months old; 40.0%). Seroprevalence to *N. caninum*, assayed by cELISA (VMRD) and confirmed by an indirect fluorescent antibody test, was low (6.8%, 4 of 59). The prevalence of *T. gondii* observed in wild cats in Majorca is one of the highest reported worldwide in this species and the highest observed in Europe to date. The results suggest that feral cats in Majorca have a high rate of *T. gondii* infection with important implications for public health on the island since the seropositive cats are likely to have already shed *T. gondii* oocysts in the environment.

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## 1. Introduction

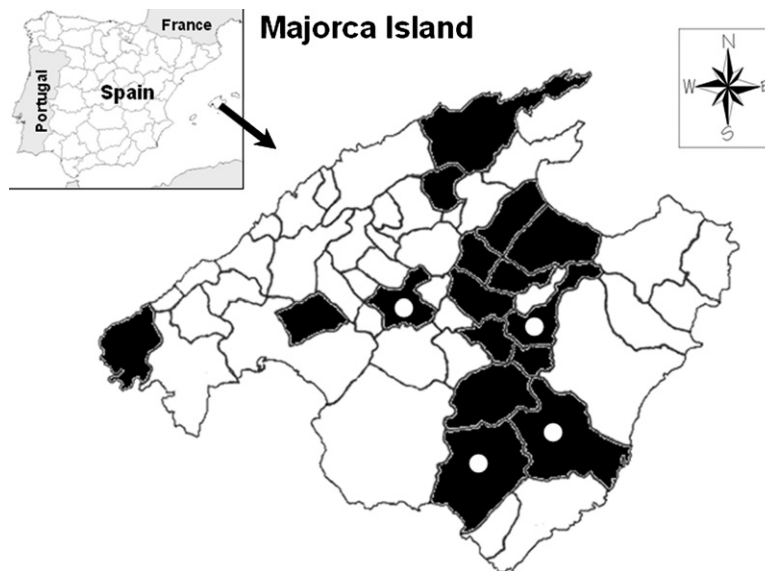
Cats are important in the epidemiology of *Toxoplasma gondii* infection because they are the only hosts that can excrete environmentally resistant oocysts in nature (Dubey, 2009). A recent review by Jones and Dubey (in press) summarized worldwide serological surveys in felids, including cats from Spain. Most of these surveys were from domestic cats in semi-urban or urban areas. There is little information from strictly wild living cats (*Felis silvestris catus*) because these cats are difficult to

catch. In one study, *T. gondii* antibodies were found in 70% of 19 feral cats that had minimum human contact from Mona Island, Puerto Rico (Dubey et al., 2007).

*Neospora caninum* is an obligate intracellular protozoan and until 1988 was misdiagnosed as *T. gondii*. Unlike *T. gondii*, canids are its definitive hosts and this parasite has not been demonstrated or isolated from cats (Dubey et al., 2007), but antibodies to *N. caninum* were reported from cats (Dubey et al., 2002; Ferroglio et al., 2005; Bresciani et al., 2007; Hornok et al., 2008; Millán et al., 2009).

Majorca is the largest of the Balearic Islands (Spain) located in the Mediterranean Sea. In the rural areas of the island, there is an abundant population of wild living feral cats [feral cat is defined as a cat that is not attached to a particular household (Liberg et al., 2000)]. These cats have

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**Fig. 1.** Map of Majorca, showing the areas where feral cats were surveyed (in black). Cats seropositive to *Toxoplasma gondii* were found in all these areas. Cats seropositive to *Neospora caninum* were found in the areas marked with a white spot.

no specific owner, are not subject to vaccination programs and do not receive prophylactic or curative treatments against parasites; their population size is unknown. These feral and other free-roaming cats are at the top of the food chain; they are exposed to a wide variety of pathogens, and have been shown to be excellent sentinels of infectious and parasitic diseases and to provide useful information on environmental contamination and circulation of pathogens in domestic and wild ecosystems (Millán et al., 2009).

We report *T. gondii* and *N. caninum* antibodies in wild living cats from Majorca Island, Spain.

## 2. Material and methods

From July to November, 2008, 59 feral cats were captured in baited traps during authorized predator control campaigns in 16 hunting estates across Majorca Island, Balearic Islands, Spain (Fig. 1). Although the inner parts of the island were more intensely surveyed, this is the zone with the higher densities of cats. Nevertheless, areas from the western, northern and southern most part of the island were also surveyed.

The climate of Majorca is temperate Mediterranean with mild temperatures in winter and summer and an average relative humidity of 70%. There were 47 adults (22 males, 25 females) and 10 juvenile (<6 months; six males, four females) cats. Sex and age were not recorded for two cats. All cats were anaesthetized with a combination of ketamine (Imalgène<sup>®</sup>, Merial, France) and xylazine (Rompun<sup>®</sup>, Bayer, Spain). Blood samples were collected from a cephalic vein and sera were stored at  $-20^{\circ}\text{C}$  until assayed.

Sera were assayed for antibodies to *T. gondii* by the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). Sera were diluted at 1:25, 1:50, 1:100, 1:200, 1:500, 1:1000 and 1:2000 dilutions. Samples with doubtful results were re-examined. A commercial positive control serum (Toxotrol A, Biomérieux, France)

diluted from 1:25 to 1:3200 (with a minimum titer of 1:200 in each test) and serum dilution buffer without serum as negative control were included in each test. A titer of  $\geq 1:25$  was indicative of *T. gondii* infection in cats (Dubey and Thulliez, 1989; Dubey et al., 1995).

A commercial competitive ELISA (cELISA) from VMRD (Pullman, Washington, USA) was used for the detection of *N. caninum* antibodies according to the manufacturers' instructions. Positive and negative control samples were provided in the kit. Percentage of inhibition (%I) was obtained by the formula:  $\%I = 100 - [( \text{sample OD} \times 100 ) / \text{mean negative control OD}]$ . If %I was more than 40% the sample was considered positive. In principle this test can be used in any species but has not been validated for *N. caninum* infection in cats. Therefore, samples positive by cELISA were confirmed by an indirect fluorescent antibody test (IFAT) using slides coated with whole *N. caninum* tachyzoites (VMRD, Pullman, Washington, USA), and FITC-labelled goat anti-cat IgG (Jackson ImmunoResearch Laboratory Inc., West Grove, Pennsylvania, USA) at 1:100 dilution. A cut-off of 1:50 or higher was used as positive.

The statistical data analysis was performed using Statistica 6.0 (StatSoft, Inc). Differences in prevalence between sex and age groups were tested by Fisher's exact test.

## 3. Results

Antibodies (MAT,  $\geq 1:25$ ) to *T. gondii* were found in 50 of 59 (84.7%, 95% confidence intervals [CI] = 0.73–0.92) cats with titers of 1:25 in 1, 1:200 in 4, 1:500 in 7, 1:1000 in 9, and  $\geq 1:2000$  in 29 cats. At least one positive animal was detected in each of the surveyed areas (Fig. 1).

Antibodies to *T. gondii* were found in 44 of 47 (93.6%, CI = 0.83–0.98) adult (>6 months old) cats and in 4 of 10 (40.0%, CI = 0.15–0.71) juvenile ( $\leq 6$  months old) cats ( $\chi^2 = 17.8$ ,  $p < 0.001$ ). Antibodies to *T. gondii* were found

in a similar number of males and females (23 of 28, (82.1%) males, and 26 of 29 (89.7%) females ( $p > 0.05$ ).

Antibodies to *N. caninum* were found in four (6.8%) cats by cELISA and results were confirmed by IFAT. All four *N. caninum* seropositive cats were captured in different areas (Fig. 1) and all also had *T. gondii* antibodies.

#### 4. Discussion

The seroprevalence of *T. gondii* observed in cats in the present study is one of the highest reported in this species worldwide and the highest reported in Europe (Jones and Dubey, in press). It is noteworthy that 38 of 59 (64.4%) cats, had high antibody titers (1:1000 or higher) and all positive juvenile cats had this level of antibodies. Whether this 64% high titer positivity is related to the strains of *T. gondii* circulating in this population of cats is unknown and merits further investigation.

It is difficult to compare different serological surveys in felids because the seroprevalence of *T. gondii* varies between feral and domestic, by the age of cats, the method of serologic testing, and the geographical location. The serological test and the cut-off level used can be compared when such data are available. Several studies have used the MAT but the end titrations were seldom done because the reagents are expensive. Among serological surveys of cats from Spain, Gauss et al. (2003) found *T. gondii* antibodies in 45% of 220 cats from Barcelona, Spain; of these only 16 (7.2%) cats had MAT titers of 1:500 or higher. Millán et al. (2009) found MAT ( $\geq 1:25$ ) antibodies in 52% of 25 feral and free-roaming cats from Andalusia, Southern Spain. In this study, the highest titer observed was 1:500 in four cats (unpublished data). Miró et al. (2004) reported 32.3% in 585 cats from mainland Spain but these cats were tested by IFAT and the cut-off was 1:80.

Prevalence of *T. gondii* infection in general is higher in feral cats that hunt for their food and have greater access to potential intermediary hosts than domestic cats (Jones and Dubey, in press). Availability of infected food and diet appears to be the main determinant criteria for *T. gondii* seroprevalence. Afonso et al. (2006) found only low (18.6%, MAT, cut-off 1:40) seroprevalence in 301 semi-urban feral cats from France. The highest seroprevalence observed previously in cats in other parts of Europe was in Ghent, Belgium where antibodies (MAT, cut-off 1:40) were found in 70.2% of 243 urban cats (Dorny et al., 2002).

The seroprevalence of feral cats to *N. caninum* was much lower than that to *T. gondii*, similar to most of the studies that compared seroprevalence in both parasites in different species (reviewed by Sobrino et al., 2008) indicating that feral cats in Majorca had more exposure in the natural environment to *T. gondii* than to *N. caninum*. The seroprevalence of *N. caninum* observed in the present study was low, as observed by Hornok et al. (2008) or Millán et al. (2009). However, antibodies were detected in cats from four different areas, indicating that *Neospora* may have a wider distribution.

In the present study, all areas surveyed had at least one *T. gondii*-infected cat, and all cats were likely born and lived permanently on Majorca Island indicating that *T. gondii* infection is likely to be endemic in other hosts on this

island. Cats are thought to acquire *T. gondii* infection by eating tissues of infected animals, and not by ingesting oocysts from the environment (Dubey and Beattie, 1988). Although the diet of feral cats in Majorcan rural areas has not been described, birds and small mammals have been considered the main sources of *T. gondii* infection. In addition, the Mediterranean climate, could favour survival of *T. gondii* oocysts shed by these wild cats. Majorca has a large sheep population that could be exposed to oocysts shed by wild cats. *T. gondii* causes abortion and neonatal mortality in sheep and the ingestion of aborted dead lambs and afterbirths could further spread *T. gondii* infection to other predators, including cats. We are not aware of any published *T. gondii* serological study among animals or humans on this island, but, 18% of AIDS patients developed cerebral toxoplasmosis in Majorca and Ibiza (Riera et al., 1995). To our knowledge, this is the first serological survey for *T. gondii* and *N. caninum* in any host on this island.

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